Targeting Trends

Reporting the latest news in Molecular Surgery

Selective deletion of CD8+ T cells by saporin-coupled MHC class I tetramers

Contributed by Paul R. Hess, Adam S. Buntzman, Sabrina L. Murray, Ellen F. Young, and Jeffrey A. Frelinger; North Carolina State University College of Veterinary Medicine, Raleigh, NC; and University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC.

CD8+ T cells constitute important effectors of the adaptive immune response, functioning principally to remove infected cells from the body, which are detected by the display of short peptides (epitopes) derived from microbial proteins within the binding groove of class I major histocompatibility complex (MHC) molecules on the cell surface. When the T cell receptor (TCR) of a primed T cell binds to its cognate peptide-MHC (pMHC) ligand, the T cell is triggered, and induces apoptosis in the infected cell. To anticipate the potential myriad of pathogen-origin peptides that might be encountered over a lifetime, a correspondingly large, diverse TCR repertoire is randomly generated, with each nascent T cell expressing thousands of identical TCRs of a single specificity. During the subsequent selection process that occurs in the thymus, most T cells bearing TCRs that inadvertently bind MHC molecules presenting “self” peptides (i.e., derived from normal proteins) are deleted prior to entering the circulation, to prevent autoimmunity; the minority of these autoreactive T cells that escape elimination are turned off by peripheral tolerizing mechanisms. In some immune-mediated conditions, such as multiple sclerosis and type 1 diabetes mellitus, normal tolerance is disabled, and autoreactive CD8+ T cells are inappropriately activated, leading to organ-specific tissue destruction and clinical signs of disease. Unfortunately, non-specific inhibition of T cell responses with immunosuppressive agents has not been particularly effective for these conditions, and such drugs carry risks of cancer and serious infections. Selective deletion of the pathogenic CD8+ T cells would appear to be an ideal strategy, but, until recently, there has been no efficient means of targeting just the culprits. In 1996, Altman et al. showed that CD8+ T cells of known specificity could be discriminated from other T cells in polyclonal populations by the use of soluble complexes, widely known as “tetramers,” consisting of four identical pMHC molecules bound to streptavidin.1 When coupled to a fluorophore, such tetramers permit ready visualization of epitope-specific T cells by flow cytometry.

(continued on page 6)

Artist’s conception of an MHC class I toxic tetramer (illustration: AM Harvey, NCSU-CVM).
This year’s winner of Poster of the Year goes to Arshad Khan of USC for his poster: *Stimulus-, circuit- and intracellular-level determinants of MAP kinase and CREB activation in parvicellular hypothalamic paraventricular neurons*. AM Khan, KL Rapp, TA Ponzie, G Sanchez-Watts, AG Watts. Dr. Khan’s work will be featured on the cover of the next issue of *Targeting Trends*.

Dr. Khan’s poster continues the researchers’ work on catecholaminergic neurons and feeding. The featured experiments involved downstream effects on signal transduction when something is missing, in this case, catecholaminergic neurons. The study followed on their work published in 2007 (*J. Neurosci*. 27:7344-7360) which asked if there were a causal relationship of noradrenergic neurons in the system. Anti-DBH-SAP (Cat. #IT-03) allowed this to be determined.

There were many great abstracts using ATS products, and this was a very difficult year for deciding the winner. Runners-up (and who knows how these selections are made!) included these fine posters.

Dale Sengelaub presented his latest work also looking at the effects of something missing: *Protection from dendritic atrophy with testosterone following partial motoneuron depletion: Timing and duration of treatment, functional correlates in motor activation*. KD Coons, DR Sengelaub. Partial loss, due to CTB-SAP (Cat. #IT-14), results in dendritic atrophy of survivor motoneurons, and this work shows administration of testosterone has a neuroprotective effect. A beautiful poster.

The poster presented by Thiago Moreira and Ana Takakura (equal contributors) continues the work from this Brazilian pair with the Guyenet laboratory, concerning the control of central chemoreflex from the retrotrapezoid nucleus: *Selective lesion of retrotrapezoid Phox2b-expressing neurons attenuates the central chemoreflex in rats*. TS Moreira, AC Takakura, RL Stornetta, PG Guyenet. SSP-SAP (Cat. #IT-11) was used to specifically delete a small number of cells, but reaching a threshold to show a behavioral effect. Ann Schreihofer was a Poster of the Year winner in 1999 when she was in the Guyenet lab.

And last, but not least, Mark Baxter of the University of Oxford, Department of Experimental Psychology, used ME20.4-SAP (Cat. #IT-15) to eliminate cholinergic neurons in the dorsolateral prefrontal cortex, resulting in difficulty of memory maintenance in feeding tasks: *Cholinergic depletion of prefrontal cortex impairs acquisition of the delayed response task in rhesus monkeys*. MG Baxter, DA Kyriazis, PL Croxson. Dr. Baxter’s molecular surgery, requiring numerous injections, was a technical *tour de force*.
Targeting Topics: Recent Scientific References

Reviewed by Matthew Kohls

Neuropathic pain is maintained by brainstem neurons co-expressing opioid and cholecystokinin receptors

It has been hypothesized that a subset of rostral ventromedial medulla (RVM) neurons co-expressing the cholecystokinin type 2 receptor and the mu-opioid receptor are responsible for the maintenance of neuropathic pain. Rats were treated with 50-ng bilateral RVM injections of Dermorphin-SAP (Cat. #IT-12), CCK-SAP (Cat. #IT-31), or saporin (Cat. #PR-01) as a control. Lesion of the RVM neurons prevented hyperalgesia in response to CCK treatment, and shortened abnormal pain states caused by sciatic nerve injury.

Cardiac Damage after Lesions of the Nucleus Tractus Solitarii

Specific neurokinin-1 (NK-1) receptor-expressing neuron lesions in the nucleus tractus solitarii (NTS) have led to the unexplained death of treated rats. In this work the authors examined cardiac specific parameters in rats after administration of 9.4 ng of SSP-SAP (Cat. #IT-11). The SSP-SAP was directed to either the dorsolateral and medial portions of the NTS, or into the brain stem outside of the NTS as a control. The data suggests that NTS lesion interrupting the baroreflex may induce cardiac arrhythmias and other myocardial changes leading to sudden cardiac death.

Endosialin protein expression and therapeutic target potential in human solid tumors: sarcoma versus carcinoma

Endosialin is an antigen expressed in many human cancer cell lines. As part of a wide-ranging study investigating clinical specimens, cell culture, and animal models, this group used Hum-ZAP (Cat. #IT-22) combined with a humanized anti-endosialin antibody in cell proliferation assays. Mouse IgG-SAP (Cat. #IT-18) was used as a control. The anti-endosialin antibody and Hum-ZAP were incubated together in equimolar concentrations then applied to cells in culture in 0.5 pM to 50 nM concentrations. Various cancers, including synovial sarcoma, fibrosarcoma, and osteosarcoma among others, were found to express endosialin.

Attentional demands for demonstrating deficits following intrabasalis infusions of 192 IgG-saporin

Attentional processing has been shown to be dependent on basal forebrain cholinergic inputs to the cerebral cortex. In this work the authors wished to specify which components should be used to demonstrate deficits following the loss of these neurons. Rats received 200 ng intrabasalis infusions of 192-IgG-SAP (Cat. #IT-01). Testing of lesioned animals indicated that attentional deficits are due to increase of overall attentional task demands as opposed to any single task parameter.

Organization of food protection behavior is differentially influenced by 192 IgG-saporin lesions of either the medial septum or the nucleus basalis magnocellularis

In this work the authors used a food-protection model to investigate the role of cholinergic neurons in the processing of information from internal and external sources. Rats received the following amounts of 192-IgG-SAP (Cat. #IT-01): 15 ng or 20 ng into the medial septum (MS), or 20 ng into the nucleus basalis magnocellularis (NB). While the NB lesions reduced the number of successful food protection behaviors, lesions in the MS disrupted the temporal organization of this behavior.

Please visit www.ATSBio.com to see a complete list of references.
Targeting Topics: Recent Scientific References
(continued from page 3)

Selective lesion of septal cholinergic neurons in rats impairs acquisition of a delayed matching to position T-maze task by delaying the shift from a response to a place strategy
Fitz NF, Gibbs RB, Johnson DA

It has been theorized that the effect of cholinergic lesions of the medial septum on learning depend on the stressful nature of the task being learned. The authors injected 0.2 µg of 192-IgG-SAP (Cat. #IT-01) into the medial septum of rats, then examined the strategies used by these animals to learn a delayed matching to position T-maze task. Lesioned animals were less able to switch from one strategy to another, indicating that this mechanism is the primary one affected by septal cholinergic lesions.

Selective lesion of medial septal cholinergic neurons followed by a mini-stroke impairs spatial learning in rats
Craig LA, Hong NS, Kopp J, McDonald RJ

Recent work has suggested that reduced levels of acetylcholine, seen in Alzheimer’s disease patients, increases the susceptibility of hippocampal neurons to future challenges. Rats received two injections totaling 7.5 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum/vertical limb of the diagonal band of Broca. The vasoconstrictor endothelin-1 was used to create small localized strokes in the hippocampus of lesioned animals. The data suggest that loss of these hippocampal neurons compromises functional recovery from stroke.

Descending facilitation from the brainstem determines behavioural and neuronal hypersensitivity following nerve injury and efficacy of pregabalin
Bee LA, Dickenson AH

Rostral ventromedial medulla (RVM) facilitatory On cells are thought to be involved in the mechanisms that control chronic pain. Dermorphin-SAP (Cat. #IT-12, 3 pmol) injected into the RVM of rats was used to examine how mu-opioid receptor-expressing facilitatory cells fit into this circuit. Saporin (Cat. #PR-01) was used as a control. The results show that activity in the RVM may influence the outcome of nerve injury.

Cholinergic depletion of the medial septum followed by phase shifting does not impair memory or rest-activity rhythms measured under standard light/dark conditions in rats
Craig LA, Hong NS, Kopp J, McDonald RJ

It has been theorized that cognitive decline observed in Alzheimer’s disease is in part due to disruption of the circadian rhythm (CR) in these patients. Some basal forebrain cholinergic neurons project to the suprachiasmatic nucleus, which is responsible for maintenance of CR. Rats received two injections totaling 7.5 ng of 192-IgG-SAP (Cat. #IT-01) into the medial septum/diagonal band of Broca. Lesioned animals did not show any evidence of CR disruption.

Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms
Goz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP

Retinal ganglion cells expressing melanopsin photopigment are thought to be involved in non-image forming visual responses to light. The authors had a custom conjugate made between saporin and an anti-melanopsin antibody. A 400-ng injection of the melanopsin-SAP (now available as Cat. #IT-44) conjugate into the eye of a mouse resulted in a loss of the targeted cells. Rabbit IgG-SAP (Cat. #IT-35) was used as a control. The data indicates that melanopsin-containing cells are involved in the response to certain non-image forming visual input.
Targeting Talk: Anti-IgM-ZAP
by Dr. Douglas Lappi

Q: We were wondering how an IgM primary antibody might work in a Mab-ZAP assay. I realize that the conjugated antibody is an anti-IgG whole molecule antibody. However there may well be aspects/epitopes shared in common between IgG and IgM that might render an IgM primary useful with the Mab-ZAP reagent… or not? Has anyone looked at this with your products?

A: We do believe, but have not confirmed, that you will see a cross-reactivity, but at a lower level. We do sell a second immunotoxin for IgM’s, Anti-M-ZAP (Cat. #IT-30) which is made from a goat anti-murine IgM.

Anti-M-ZAP
(Cat. #IT-30)
A "second" immunotoxin that relies on your antibody for cytotoxicity to target cells.

Elimination of Specific Cell Type
• Cells that internalize your mouse monoclonal IgM antibody will be eliminated.

• Potency may vary according to the specificity and affinity of YOUR antibody to ITS receptor.

• Anti-M-ZAP is most effective in determining specificity of your antibody and suitability for conjugation as a primary immunotoxin.

Targeting Teaser Winners
The solution to the puzzle was:

Jumbles: AMOEBA GRANULAR PROTEIN CULTURE INCUBATOR

Answer: The . . . BIG PICTURE

Congratulations to the puzzle solvers from our last newsletter. Each winner receives $100 credit towards research product purchases from Advanced Targeting Systems.

WINNERS: Glenn Kageyama, Cal Poly Pomona Univ, Pomona, CA  * Sophie Lopen, Methodist Hospital Res Inst, Houston, TX  * Caroline Kent, Mayo Clinic, Jacksonville, FL  * Aamir Ahmad, Karmanos Cancer Inst, Detroit, MI  * Kimuda Saraff, Cal State Univ, Northridge, CA  * April Price, Univ California, San Francisco, CA  * Thea Marlinga, Libertyville, IL

Anti-M-ZAP
(Cat. #IT-30)
A "second" immunotoxin that relies on your antibody for cytotoxicity to target cells.

Elimination of Specific Cell Type
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Feature Antibodies

NGFr (mu p75) Rabbit Polyclonal
Cat. #AB-N01, 100 µl or affinity-purified Cat. #AB-N01AP, 50 µg
Recognizes p75NTR in mouse. The antisera was developed in rabbit using an extracellular fragment from the mouse p75 receptor (amino acids 43-161). The antibody was affinity-purified using the extracellular domain of p75.

Applications: immunohistochemistry (frozen or paraffin-embedded cells and tissue; 1:150), immunoprecipitation, immunoblotting (1:2,000), flow cytometry (1:1,000), and blocking the function of NGFr (1:1,000).

NGFr (ME20.4, p75) Mouse Monoclonal
Cat. #AB-N07, 100 µg
Recognizes the p75NTR (low affinity neurotrophin receptor) in human, primate, rabbit, sheep, dog, cat, and pig. It was derived from immunization of mice with WM245 melanoma cells.

Applications: flow cytometry (1:100), immunoprecipitation, immunohistochemistry (frozen), electron microscopy (1:200), immunocytochemistry (10 ng/ml), and radioimmunoassay.

Upcoming Events
Experimental Biology
April 18-22
New Orleans, LA
Booth #218

Amer Assoc Cancer Research
April 18-22
Denver, CO
Booth #1760
Selective deletion of CD8+ T cells by saporin-coupled MHC class I tetramers (continued from page 1)

We and others have shown that, after binding to the TCR, tetramers are endocytosed by the T cell. These two characteristics – specific binding and rapid internalization – suggested that tetramers might be a useful way to selectively deliver an intracellularly-active toxin to pathogenic T cells. To investigate this hypothesis, we used TCR-transgenic P14 mice as a source of CD8+ T cells, which recognize a viral glycoprotein-derived peptide, gp33, presented by the class I MHC molecule, H2-Db, and bind to the tetramer, gpC9M. To confirm our observations with a second epitope, we employed TCR-transgenic HY mice, whose CD8+ T cells bind to the H2-Db tetramer, hyC2A. Toxic tetramers were generated from gpC9M and hyC2A pMHC monomers using streptavidin-saporin (SA-SAP; Cat. #IT-27). After assembly, these tetramers retained the TCR-binding specificity of their fluorophore-labeled counterparts, and inhibited translation in a cell-free assay as potently as parent SA-SAP alone. To determine whether T cells would efficiently internalize SAP-coupled tetramers, we briefly cultured quiescent P14 T cells with the gpC9M-SAP tetramer, or as negative controls, with non-toxic gpC9M (not shown) or toxic hyC2A-SAP tetramers. Following the addition of FITC-labeled SAP antibody (Cat. #FL-02), T cells were subsequently incubated at either 37°C or 4°C, which permitted or prohibited endocytosis, respectively. To discriminate internal and external fluorescence, tetramer-antibody-fluorophore complexes on the surface were either removed with an acetic acid solution ("stripped"), or allowed to remain intact ("washed"), prior to analysis. As shown in Fig. 1, acid-resistant fluorescence (gray line), corresponding to endocytosed SAP, was found in all metabolically-active P14 T cells incubated with the cognate gpC9M-SAP, but not with control tetramers. We next evaluated the ability of the SAP-coupled tetramers to kill T cells in vitro. Purified P14 and HY T cells were incubated with either non-toxic tetramers alone; non-toxic tetramers plus free (unbound) SAP; or SAP-coupled relevant or irrelevant tetramers. Surviving cells were identified by exclusion of a membrane-impermeant dye, 7-aminoactinomycin-D, at the time points shown in Fig. 2A. Some cell loss was observed over time with all treatments, characteristic of stimulated, cultured T cells; however, incubation of HY T cells with hyC2A-SAP resulted in the death of 98% of cells within a 3-day period. Free SAP was not toxic to T cells. In vitro killing of these CD8+ T cells by the cognate SAP-coupled tetramers depended on the tetramer dose (Fig. 2B), and the avidity of the tetramer-TCR interaction (not shown).

We then sought to determine if our toxic tetramers could delete specific CD8+ T cells in vivo. Fluorophore-labeled tetramers, when injected intravenously, rapidly bind to cognate T cells in lymph nodes, spleen, and bone marrow, suggesting that SAP-coupled tetramers similarly should be able to reach their targets. To test this hypothesis, we transferred P14 T cells into recipient mice, and after 24 h, administered either gpC9M or gpC9M-SAP. Fig. 2C shows that, after 3 d, >75% of P14 cells were deleted from the spleen in gpC9M-SAP-treated mice; the recovery of control HY cells was not different between treatment groups. At this dosage, injection of the SAP-coupled tetramers caused an acute, mild liver injury, but mice showed no clinical signs of illness.

These studies showed that tetrameric pMHC complexes can be used to deliver a potent toxin, SAP, to epitope-specific CD8+ T cells in vitro and in vivo, leading to deletion of the target population. Such toxic tetramers could represent a novel and effective means for eradicating pathogenic T cell responses in selected immune-mediated diseases.

References:
Targeting Tools: Assays from Cytometry Research

Cancer Cell Lines — Compound Screening

The CR-60 panel is a set of 59 human cancer cell lines derived from diverse tissues; brain, blood and bone marrow, breast, colon, kidney, lung, ovary, prostate and skin. Since 1992, researchers from across the world have subjected these cell lines to a battery of experiments including extensive pharmacological characterization by treatment with over 100,000 chemical compounds, chromosome karyotyping and gene expression analysis. Within the 59 lines that make up the CR-60 set there are three pairs of lines with high identity to each other (NCI-ADR-RES/OVCAR-8, M14/MDA-MB-435 and SNB19/U251) suggesting the members of each pair share a common source.

The CR-60 panel comprises the following tumor cell lines:

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<th>CNS</th>
<th>Non-small cell Lung</th>
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Gangsta steps in as Guest Editor
“Love the pictures (Doug looks terrific!). Love the posters. Just a little too much white space.”
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SHAMEMINC

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